Alaska Hatchery Research Group **Technical Document:**[1] 7

Title: Thermal Mark Recovery Procedures of the ADF&G Mark, Tag and Age Laboratory **Version:** 1.0

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Abstract

The Alaska Hatchery Research Program is designed to answer questions regarding concerns that hatchery fish released by private non-profit corporations in Prince William Sound (pink and chum salmon) and in Southeast Alaska (chum salmon) may have a detrimental impact on the productivity and sustainability of natural stocks. The study that was designed to answer these questions requires that samples and data collected by a contractor and by various Alaska Department of Fish and Game laboratories be combined to test hypotheses. One aspect of critical to this study is examining salmonid otoliths for the presence or absence of a thermal mark. This technical document describes the procedures used by the Alaska Department of Fish and Game, Mark Tag and Age Lab for thermal mark recovery. Procedures for thermal mark recovery include cleaning otoliths and trays, tracking trays and otoliths, otolith preparation (slide labeling, mounting the otoliths), grinding a prepared otolith to the core so that thermal mark presence or absence and thermal mark identification can be determined, and entering results to a database.

Background of AHRP

Extensive ocean-ranching salmon aquaculture is practiced in Alaska by private non-profit corporations (PNP) to enhance common property fisheries. Most of the approximately 1.7B juvenile salmon that PNP hatcheries release annually are pink salmon in Prince William Sound (PWS) and chum salmon in Southeast Alaska (SEAK; Vercessi 2014). The large scale of these hatchery programs has raised concerns among some that hatchery fish may have a detrimental impact on the productivity and sustainability of natural stocks. Others maintain that the potential for positive effects exists. To address these concerns ADF&G convened a Science Panel for the Alaska Hatchery Research Program (AHRP) whose members have broad experience in salmon enhancement, management, and natural and hatchery fish interactions. The AHRP was tasked with answering three priority questions:

[1] This document serves as a record of communication between the Alaska Department of Fish and Game Commercial Fisheries Division and other members of the Science Panel of the Alaska Hatchery Research Program. As such, these documents serve diverse ad hoc information purposes and may contain basic, uninterpreted data. The contents of this document have not been subjected to review and should not be cited or distributed without the permission of the authors or the Commercial Fisheries Division

I. *What is the genetic stock structure of pink and chum salmon in each region (PWS and SEAK)*?;
II. *What is the extent and annual variability in straying of hatchery pink salmon in PWS and chum salmon in PWS and SEAK*?; and
III. *What is the impact on fitness (productivity) of natural pink and chum salmon stocks due to straying of hatchery pink and chum salmon*?

Introduction

To answer the above questions, we need to know the origin and pedigree of each fish captured in select streams across multiple generations. Origin refers to the type of early life-history habitat (hatchery or natural) that a fish experienced. Pedigree refers to the family relationship among parents and offspring. 'Ancestral origin' refers to the origin of an individual's ancestors (e.g., two parents of a single origin [hatchery/hatchery or natural/natural] or two parents of mixed origin [hatchery/natural]). These ancestral origins can be determined by combining information from three sources: identification of hatchery origin from otolith thermal marks, pedigree from genetic data, and age from scales (SEAK chum).

Question: How will we identify hatchery origin from otolith marks?

Salmonid otoliths are thermal marked by exposing them to repeated temperature cycles to create patterns of optically-dense bands (Volk et al. 1990). Because these can be applied accurately and identified quickly (Hagen et al. 1995), thermal marking (Figure 1) is an effective tool providing simple identifiers for hatchery salmon (Munk and Smoker 1991; Volk et al. 1990).

The North Pacific Anadromous Fish Commission (NPAFC) Working Group on Salmon Marking (WGOSM) coordinates the application of otolith mark patterns for hatchery-origin fish released in the North Pacific Rim countries; they work to minimize duplication of marks among release groups. Thus, thermal marks recovered from adult salmon can be used to identify release location of chum salmon releases in SEAK. The MTA Lab examines otoliths for thermal marks to identify origin of chum salmon in SEAK.

Goal

This technical document describes the procedures used by the MTA Lab for AHRP thermal mark recovery.

Methods

Sample types

For the AHRP project, there are two types of samples collected based on the surveys conducted: stream and pedigree. Samples collected at stream sites contain only otoliths. Samples collected at pedigree sites include DNA tissue and otoliths. Otoliths collected from stream sites are placed into a shallow 96-well tray. When pedigree sites are sampled, an otolith pair and a tissue specimen are placed into the same cell of a 48 deep-well tray. The cells in a pedigree sample tray are filled with ethanol to preserve tissue.

For each fish sampled, both right and left sagittal otoliths are removed and placed in the appropriate trays. If enough samples to fill the tray are not obtained on a sampling trip, then some wells are left empty. If otoliths are lost in the field, missing ones are represented by glass beads. Thus, when one otolith is missing, a well contains one otolith and one bead to indicate a missing otolith. A well with no otoliths contains two beads, indicating that both otoliths are absent.

Prior to shipment of stream samples to the MTA Lab, stream trays are dried so that the otoliths remain within tray wells. If otoliths are wet, they may stick to the tray lid rather than stay in place. Trays are dried by leaving them uncovered overnight. To keep the otoliths in position, two acetate compression plates taped together with double-sided tape are placed between the tray and lid. The tray, compression plates, and lid are secured with three fresh #64 rubber bands (Figure 2). Only new rubber bands are used, because old rubber bands warp, break, crack, and stretch allowing otoliths to move out of place. Prior to shipment of pedigree trays to the Gene Conservation Lab (GCL), samples were refreshed with ethanol. Processing of of pedigree trays follows methods detailed in Appendix A. After processing, the 48 deep-well tray (now only contains otoliths) are uncovered, dried, recovered, and shipped to the MTA Lab. The duplicate plate (contains only heart tissue) is uncovered, dried, recovered, and archived until DNA extraction. Archived location is entered into the GCL database, LOKI.

MTA Lab Procedures

Otolith processing procedures for the AHRP project begin with collection of stream and pedigree samples in SEAK streams and genetic tissues removed at the Gene Conservation Lab (Figure 3). These samples are then shipped to the MTA Lab where the trays are cleaned, logged in to an Oracle database to digitally track the samples, and mounting them to glass slides for reading. The mounted otoliths are ground and examined for thermal mark presence and identification by two independent readers, and any conflicts are resolved. Two independent reads are used to assess the accuracy of thermal mark presence and identification (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document). Mark recovery results are summarized on a public website, queried through the database, and reported by the AHRP project contractor.

Otolith cleaning and tray review

Upon receipt at the MTA Lab, the crew leader reviews the labels to ensure that the data recorded on the tray are legible and match the corresponding tray inventory. Discrepancies are resolved by contacting the contractor. Otoliths and trays are rinsed with a 5% chlorine solution to clean and bleach the otoliths. Trays are subsequently rinsed 0.7% thiosulfate and water to stop the bleaching process. Cleaning removes remaining tissue; otherwise, this tissue may prevent adherence to the petrographic slides or it may obscure visibility of the otolith core. The wells in each tray are checked for missing otoliths, and glass beads are added to represent absent specimens (Figure 4).

Otolith tray log-in

All data associated with each tray are entered into the Southeast Mark-recapture (SEMR) database using custom data entry applications. The data includes tray number, species, life stage, statistical week, source, gear type, location, and stream code. The number of otoliths in each tray is recorded by selecting the last well position. For pedigree trays, an additional number (deep-well plate identification number) is recorded to coordinate genetics data with otolith data. Other information on the tray label, such as collectors, comments, and shipping method, is also entered. After samples are entered into the database, each fish can be located using sample, specimen, tray, and well number.

Otolith mounting

After trays are logged-in, labels with a unique bar code are printed and affixed to one-by-two inch petrographic glass slides. The labels contain information for quick reference, such as tray, well, sample, and specimen number (Figure 5). Maintaining proper tray orientation while mounting otoliths keeps specimens in order, which is important because otolith data are associated with other information, such as genetics and scale-age data.

Trays from stream sites, which hold 96 otoliths, are positioned so that the white, pre-painted corner (painted before a project begins) is to the upper left, indicating the starting position (Figure 4). This ensures that the correct otolith is removed from the correct well. Otoliths are removed from left to right by rows. Thus, the first otolith is removed from well "A1" in row "A" and the next otolith is removed from well "A2." This continues until all otoliths are pulled and mounted from row "A"through well "A12." Once complete, otoliths are removed from the next row down starting with well "B1."

Trays from pedigree sites, which have 48 deep wells, are placed in an apparatus designed to ensure proper tray orientation and allow only one space to be open for otolith selection from the tray at one time. This apparatus is helpful because otoliths are harder to see in a deep-well plate than in a shallow 96-well tray. A notch on the bottom left corner provides a visible reference starting position (Figures 6 and 7), and these trays are oriented differently. Otoliths are pulled from top to bottom by columns. Thus, after the first otolith is removed from well "A1," the technician removes and mounts otolith from well "A2" continuing until all otoliths are pulled from the first column, column "A." Once complete, otoliths are pulled from the next column starting with "B1" and so on.

After the correct otolith is selected, the left otolith is mounted on the un-labeled side of the glass slide sulcus-side up (Figures 5 and 8) with thermoplastic cement, so that the label is protected when the otolith is ground. The right otolith remains in the tray for age and brood year determination, if necessary and is available to be used if the left otolith is unreadable. Mounted slides are stored in 100 specimen slide boxes labeled with district, subdistrict, species, sample date, statistical week, sample number, and box number. After mounting, otoliths are handled by box; the sample and box numbers on the box label are used for assessment of otolith mark recovery reads (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document).

Otolith Preparation and Mark Recovery

Prior to reading chum salmon otoliths, all readers review and study examples of thermal marks expected to be recovered during that sampling period. For this project, these marks include chum salmon released in Southeast Alaska from brood years that correspond with fish returning at age 0.2, 0.3, 0.4, and 0.5 (European age notation) in each recovery year. Please see the "Personnel Training" section of the Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document for a description of the pre-season thermal mark review process.

To examine a salmonid otolith for the presence or absence of a thermal mark, a reader first enters the specimen number directly into the SEMR Oracle database by scanning the bar code on the slide label. This connects the reader to that record in the database. Once the specimen number is entered, the reader grinds the otolith using a variable speed grinder and 800 grit grinding paper until the primordia at the otolith's core are visible under 200 x magnification on a compound microscope. If needed, the otolith can be fine-polished manually on wet nine µm grinding paper to enhance growth patterns at the otolith's core. The reader then places the slide otolith side up on a compound microscope and examines it using the 25 x and 40 x objectives to determine whether the otolith is thermal marked (hatchery-origin) or not marked (natural-origin). The reader enters the result in the SEMR Oracle database using a touch screen monitor and a custom data entry application. If a specimen is thermal marked, the reader enters the hatch code (unique thermal mark pattern), thermal mark identification (a name assigned to each hatch code that provides information regarding brood year and release site), and age (ADF&G 2011). All specimens receive a status code (readable or not readable). This status code is also used to track progress on a project. If a specimen is not readable, a reader enters a code providing a reason why an otolith could not be examined (e.g.; no otolith, crystalline, morphology problem, over-ground, or wrong species). Once a specimen is read, the slide is placed back in the slide box and stored in the MTA Lab. Accuracy of results are assessed using a variety of methods, all of which include independent re-examination of ground otoliths (see Thermal Mark Recovery Data Quality Assurance and Quality Control Technical Document).

Otolith archives

All thermal mark data processed at the MTA Lab, including the reference collection and adult recoveries, are housed in the SEMR database (Frawley et al. 2015 for details regarding AHRP data flow).

Reporting

Thermal mark read results are reported as follows:

(1) A public report, which includes the number of otoliths received, prepared, and read, the number marked, the number unmarked, and the mark identifications. Data are listed by fishery name, species, source, statistical week, statistical area, stream code, harvest type, sample date, gear, and survey site. This report can be accessed and generated via the web at: http://mtalab.adfg.alaska.gov/OTO/reports/MarkSummary.aspx

(2) Results stored in the SEMR Oracle database are integrated with results from other ADF&G labs and the project contractor in an ADF&G statewide data warehouse. This data flow between the contractor, who collects the specimens and records the sampling event data, and the MTA Lab is described in the AHRP Data Flow Technical Document (Frawley et al. 2015).

(3) Specialized reports can be developed using Microsoft Access to query the SEMR Oracle database. This is utilized for data quality control or specific reporting.

References

ADF&G. 2011. Mark Summary Report. Juneau http://www.taglab.org/OTO/reports/VoucherSummary.asp

Frawley, T. R., E. Lardizabal, and S. Johnson 2015. Alaska Hatchery Research Program Technical Document 10: AHRP Data Flow

Hagen, P., K. Munk, B. W. Van Alen, and B. White. 1995. Thermal mark technology for inseason fisheries management: a case study. Alaska Fishery Research Bulletin 2(2):143-155.

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Vercessi, L. 2014. Alaska salmon fisheries enhancement program 2013 annual report Alaska Department of Fish and Game, Anchorage http://www.adfg.alaska.gov/FedAidPDFs/FMR14-12.pdf

Volk, E. C., S. L. Schroder, and K. L. Fresh. 1990. Inducement of unique otolith banding patterns as a practical means to mass-mark juvenile Pacific salmon. American Fisheries Society Symposium 7:203-215.

Figures

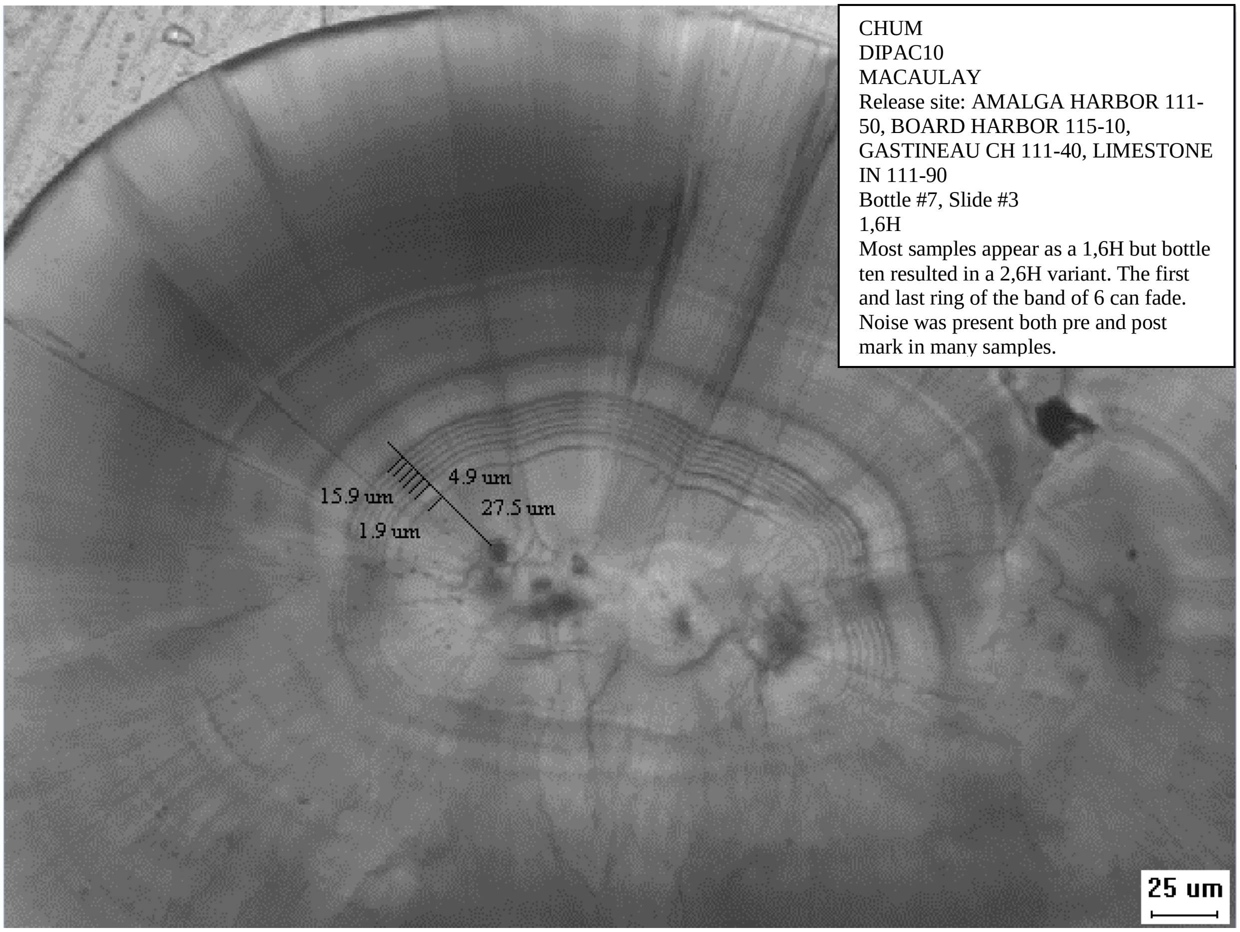

Figure 1. Image of a thermal mark from a voucher specimen. This mark is from Macaulay Hatchery, brood year 2010, and has thermal mark identification “DIPAC10.” It has a thermal hatch code of 1,6H. This hatch code indicates that from the otolith’s core there is a band with one dark ring, a space, followed by a band of six rings, prior to the hatch mark (the blurry, wider dark area beyond the thermal mark). Measurements on the annotated transect line include the distance from the otolith’s core to the first band, the width of the first band, the space between the first and second bands, and the average distance between rings in each band. All thermal mark images are published online and are available through the North Pacific Anadromous Fish Commission (NPAFC) Working Group on Salmon Marking (WGOSM) website:
http://wgosm.npafc.org/MarkSummary.asp

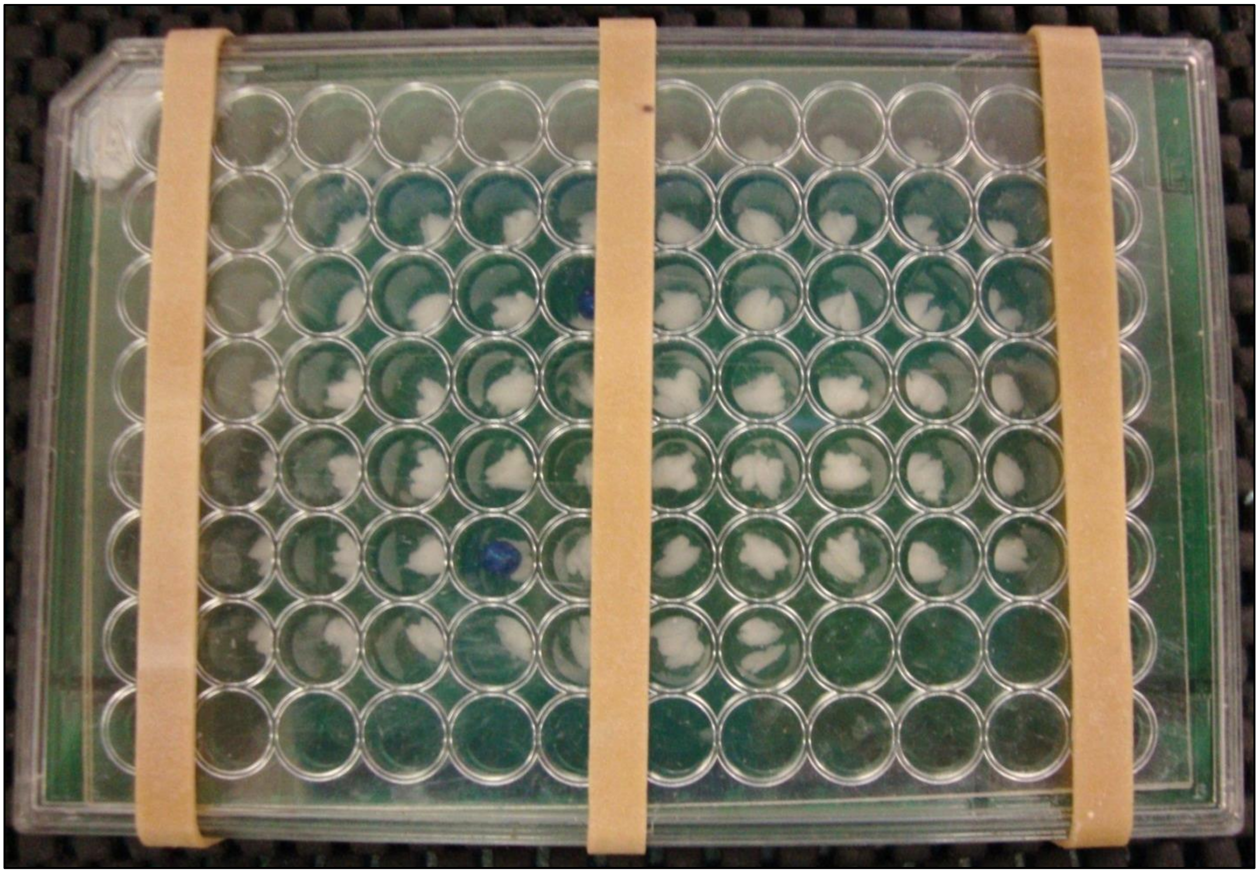

Figure 2. Otolith tray from a stream sampling site prepared for shipment. Tray includes two acetates taped together and placed between the tray and lid. Lid is secured with three “fresh” #64 rubber bands. Note the white paint added to notched corner (upper left) to aid in identifying correct orientation of tray.

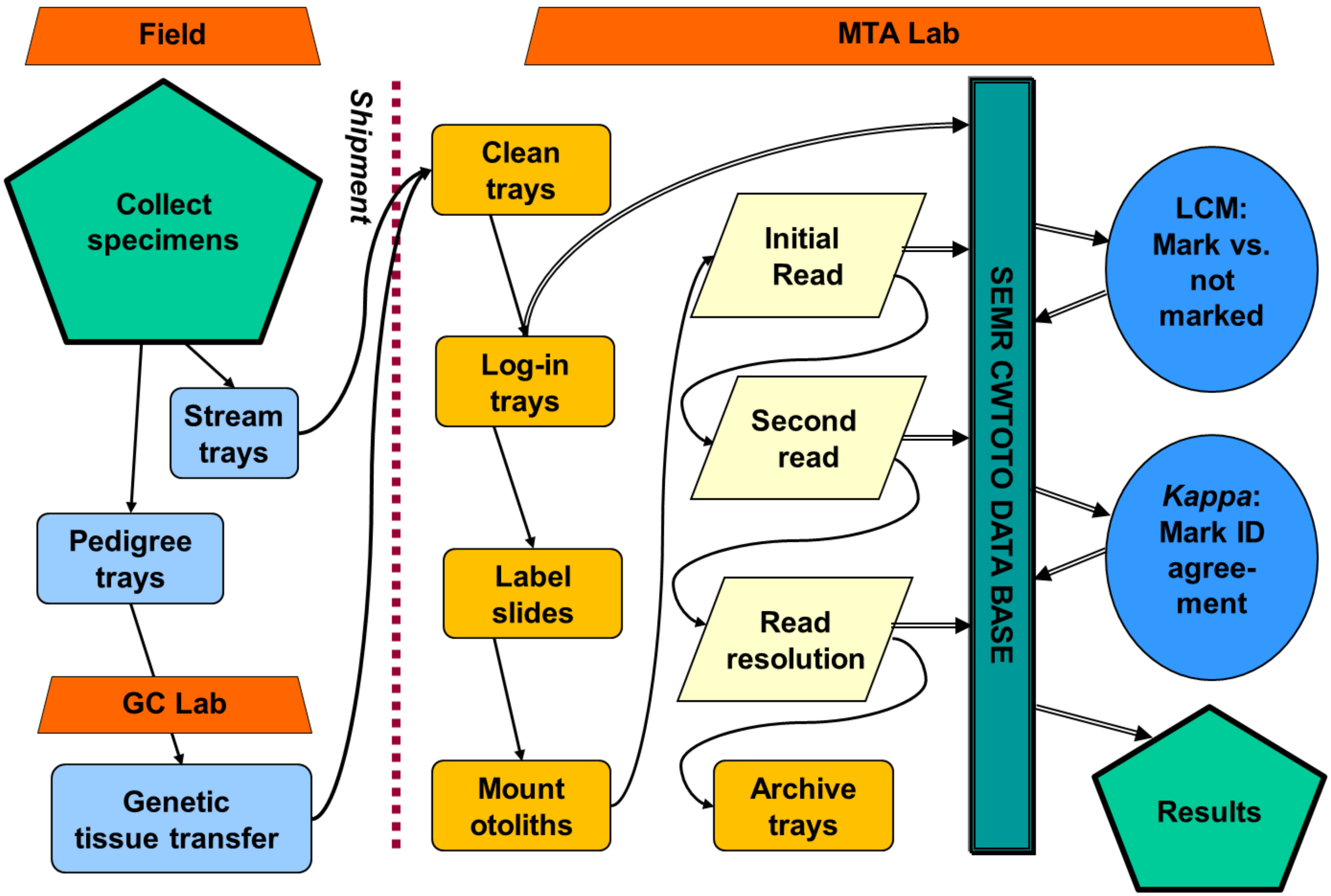

Figure 3. Flow diagram of Southeast Alaska otolith processes for the Alaska Hatchery Research Program. Shapes indicate different processes: hexagons are the beginning and end of flow, trapezoids are location of process, rounded rectangles are tray or otolith preparation, parallelograms are otolith data collection, ovals are statistical examination of results. Solid arrows indicate specimen flow, double arrows indicate data flow. See text for descriptions of each process and the QA/QC technical document for descriptions of statistical methods. LCM: Latent class model, *Kappa* is Fleiss's *Kappa* statistic. Both are usedfor QA/QC.

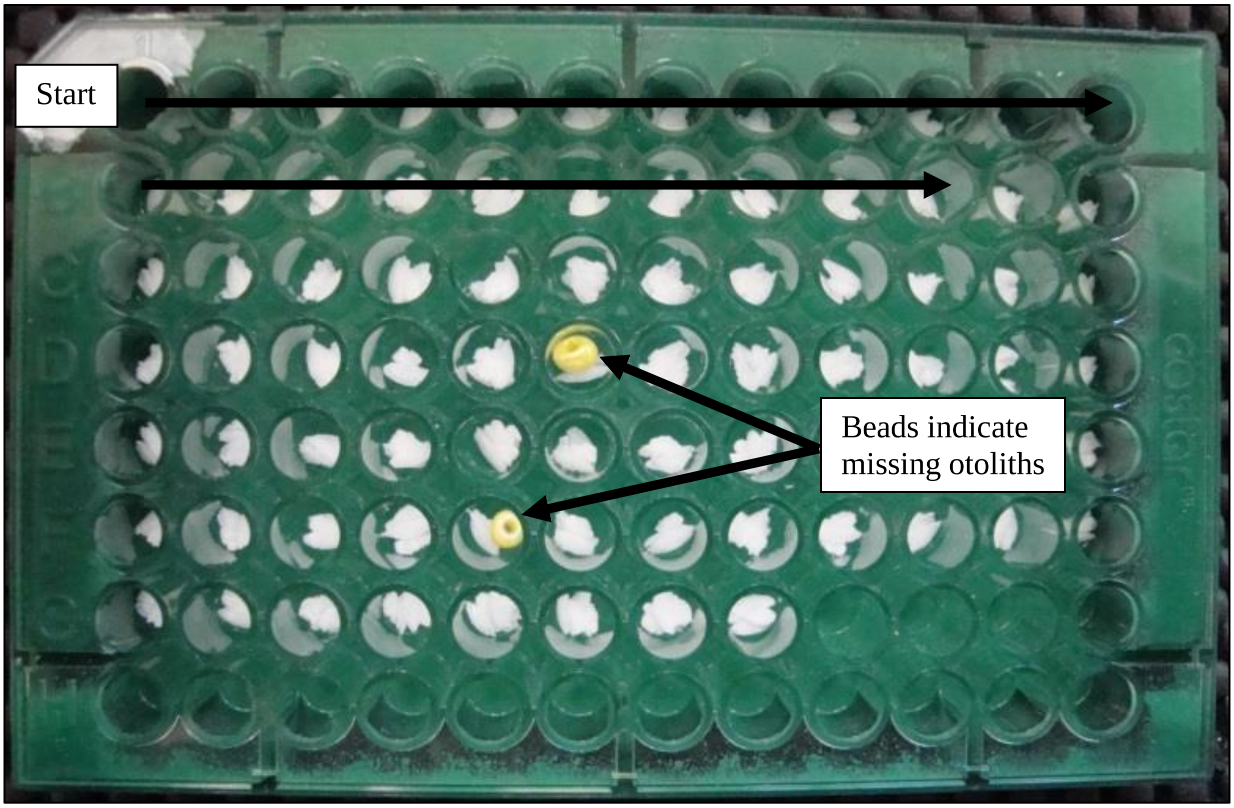

Figure 4. Illustration of otolith placement in a tray from a stream sampling site. The tray is positioned so that the white, painted notch is in the upper left corner. Raised letters are visible on the left side of the tray; numbers are viewable across the top of the tray. Otoliths are added left to right by rows. Thus, samplers fill the first well with an otolith pair (A1, then A2). Beads indicate missing otoliths.

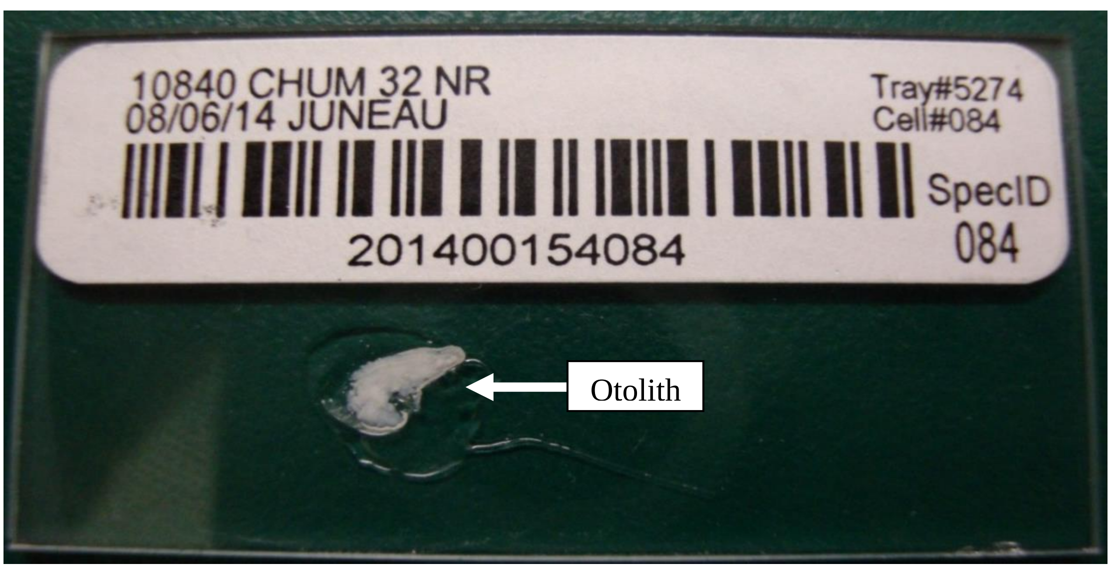

Figure 5. Example of a petrographic glass slide (1 x 2 in) labeled with a unique bar code. Slide shows a left otolith mounted to the back using thermoplastic cement. Information includes: sub-district (108-40), statistical week (32), sample date (8/6/14), species (chum), tray number (5274), cell number (084), sample number (201400154), and specimen number (084).

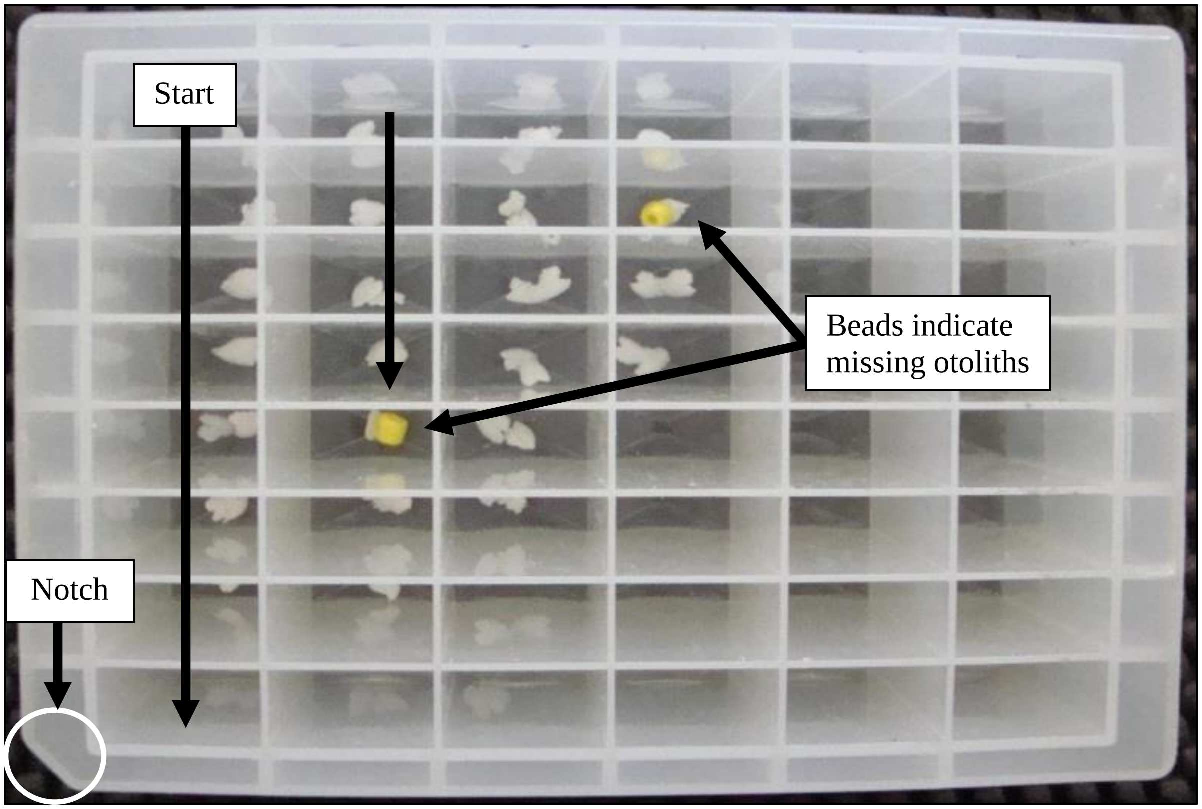

Figure 6. Otolith location in a deep-well plate used to collect pedigree stream samples. The tray is positioned so that the notch is in the lower left corner. Otoliths are placed top to bottom by columns. Thus, the first otolith is in the upper left well, and second otolith is in the well below the first otolith. Beads indicate missing otoliths.

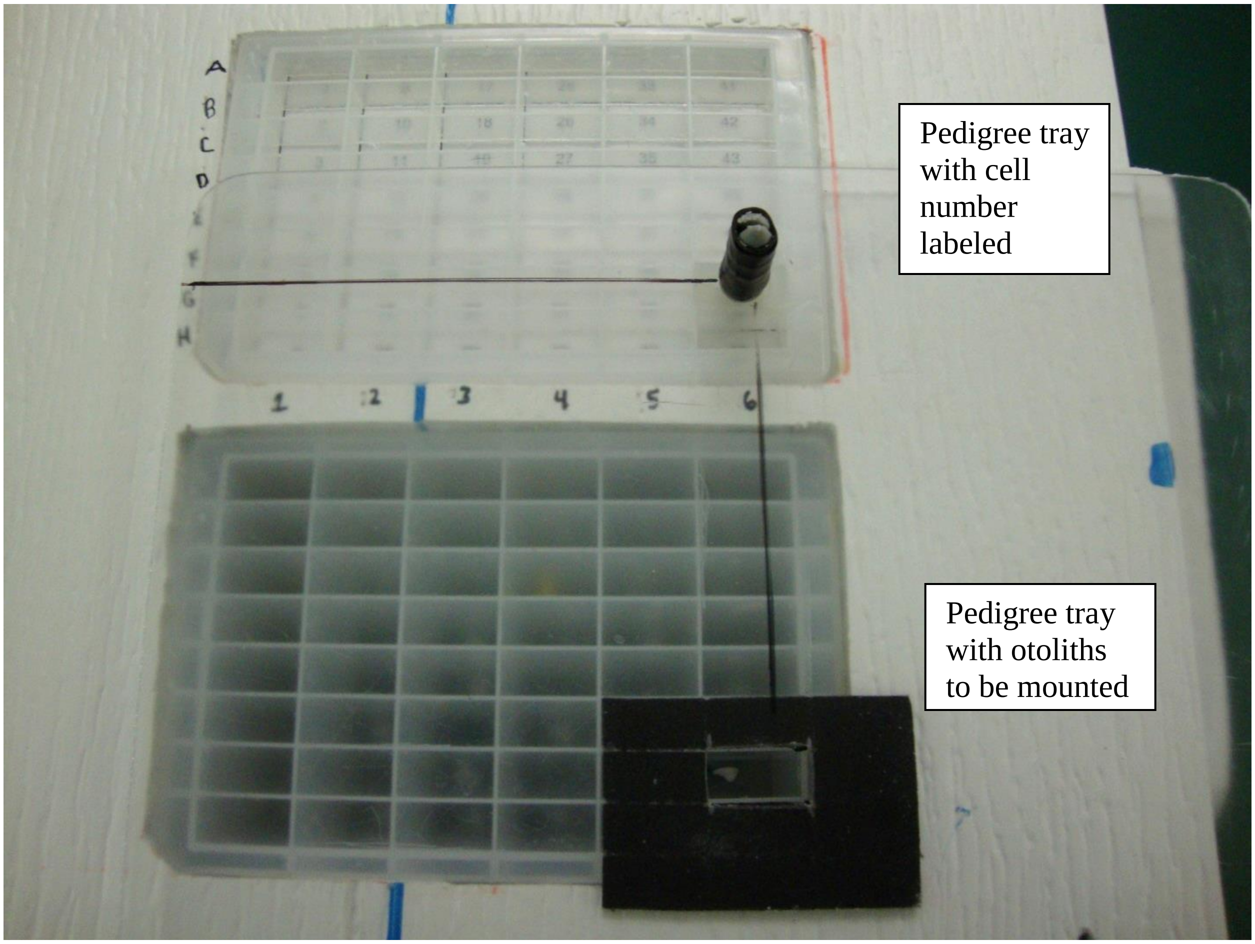

Figure 7. Mounting apparatus for pedigree stream deep-well plates to ensure the correct otolith is selected. The apparatus permits only one cell number and well position open at a time.

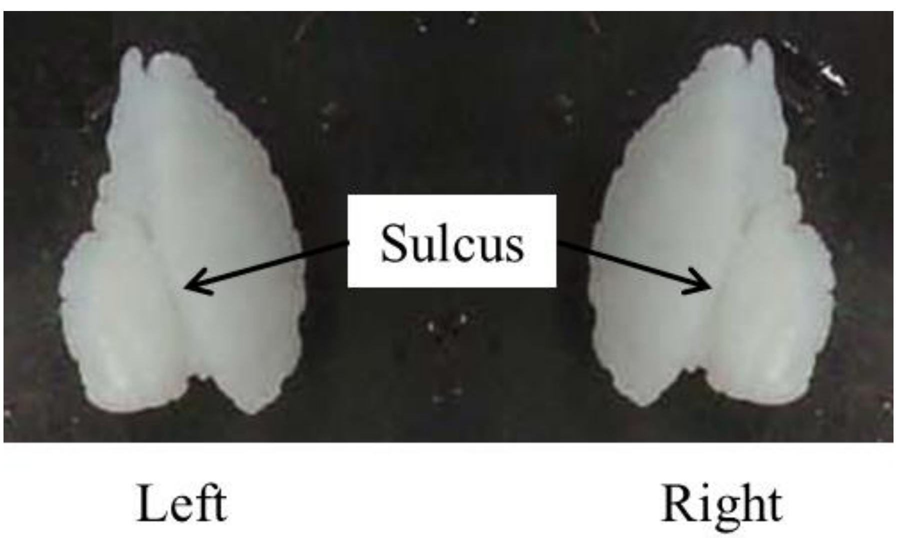

Figure 8. Left and right sagittal otoliths, sulcus side up.

Appendix A. Tissue Transfer Protocol for 48 Deep-Well Plates

Setup:

1. Mark each original 48 well plate mat using solvent resistant marker with information from the plate: (a) project name and (b) plate number. Label a duplicate plate with an identical barcode label.
2. Mark position 1 (A1) of the original mat with a marker, so mat is returned to original plate with the same orientation after the transfer is complete.
3. Ensure you have a clean split-mat cover for each plate (Figure 1).

Transfer:

1. Remove mat from original plate and set aside.
2. Use the 48-well-plate-transfer guide to set up the original and duplicate plates (Figure 1)
    a. Guide will automatically orient both plates with the notch key.
    b. Have a colleague double-check that the labels match.
3. Position the sliding white cover with the rectangular opening over position 1 (A1): the guide will automatically position on A1 in the corresponding plate. Cover columns 3–6 on both plates with the split-mat cover.
4. Proceed with transferring the genetic tissue to its corresponding well in the duplicate plate
    a. Visually confirm that an otolith is not stuck to the genetic tissue. If not sure, gently rinse the tissue with ethanol over the original well before depositing tissue into the duplicate plate.
5. Continue transferring each genetic tissue, repositioning the sliding white cover over each well to ensure accuracy of transfer, moving down A1, B1, C1, etc. before proceeding to the next column. (See Figure 2 for example of E1 setup).
6. For each well in row H, the sliding white cover will need to be flipped so that the cover's keys fit into row G wells. These keys keep the guide from sliding.
7. Continue transfer proceeding down and over columns 2–6, repositioning the split-mat covers on either side of the active columns until the plate is complete. (See Figure 3 for example of C3 setup)
8. Replace mats on both duplicate and original plate (in the same orientation as before).

64 Figure 1. 48-well-plate-transfer-guide

Figure 2. Example setup for tissue transfer from well El.

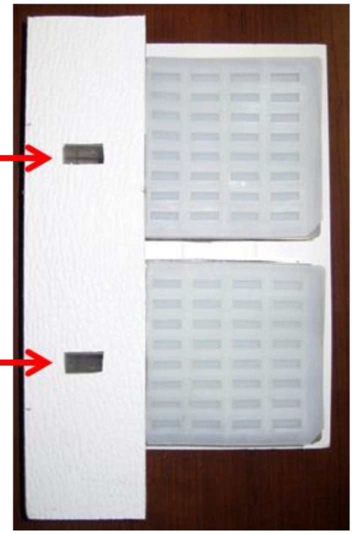

Figure 3. Example setup for tissue transfer from well C3.